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THE QUANTITATIVE ESTIMATION OF THE RENNET-ZYMOGEN:

Its Diagnostic Value in Certain Diseases of the Stomach.

BY

JULIUS FRIEDENWALD, A.B., M.D.,

CLINICAL PROFESSOR OF DISEASES OF THE STOMACH, COLLEGE OF PHYSICIANS AND SURGEONS, BALTIMORE.

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THE QUANTITATIVE ESTIMATION OF THE RENNET-ZYMOGEN; ITS DIAGNOSTIC VALUE IN CERTAIN DISEASES OF THE STOMACH.[1]

BY JULIUS FRIEDENWALD, A.B., M.D.,
CLINICAL PROFESSOR OF DISEASES OF THE STOMACH, COLLEGE OF PHYSICIANS AND SURGEONS, BALTIMORE.

THE detection and the estimation of free hydrochloric acid in the gastric contents are of considerable importance in the diagnosis of diseases of the stomach, but conclusions based upon these facts alone may be wholly fallacious, as it is well known that mental conditions and various external influences, as well as disturbances of the circulation, influence the secretion of hydrochloric acid. Not only marked changes in the mucous membrane, therefore, but also functional disturbances may cause diminution or absence of hydrochloric acid.

Quantitative examinations for free hydrochloric acid give us but little knowledge of the degree of destruction of the gastric mucous membrane, for, while free hydrochloric acid is usually entirely absent in marked change of the mucous membrane, we frequently find the same condition when the

[1] Read at the annual meeting of the Medical and Chirurgical Faculty of Maryland, April 24, 1895.

changes are but slight. It is, therefore, generally admitted that, in order to draw proper conclusions concerning the true condition of the stomach from examinations for free hydrochloric acid, the examinations should be frequently repeated. But even then we may still be in doubt as to whether there is a nervous anacidity, slight or marked catarrh, or a hyperemic condition of the stomach, secondary to diseases of some other organ.

While such variations are found in regard to the secretion of hydrochloric acid, the secretion of the ferments, or rather of their zymogens, bears a definite relation to the pathologic changes present. Boas has shown that external influences, as well as congestive conditions, have little effect on the secretion of these substances, and that a marked diminution in their quantity is aways indicative of some serious gastric lesion. The quantitative estimation of pepsin and its proenzyme has, until recently, been attended with great difficulty. Methods have been devised for this purpose by Boas,[1] Johannessen,[2] and others, but exact results cannot be obtained by any of these methods. It is, therefore, necessary, in exact examinations, to test the peptic strength by weighing the fibrin before, and then again after partially digesting it in a given quantity of gastric juice.

This method is quite complicated and wholly unsuited for practical purposes. A new method for

[1] Boas: Allgemeine Diagnostik und Therapie, 3. Auflage, S. 187.

[2] Johannessen: Studienüber die Fermente des Magens, Zeitschrift für klin. Medicin, Bd. 17, H. 3 und 4.

the estimation of pepsin, which is simple and seems to give exact results, has recently been devised by Hammerschlag.[1] This method has not as yet been generally employed.

The estimation of the rennet-ferment (lab) and its proenzyme (labzymogen) is very simple. The method usually employed is that of Boas:

The detection of the milk-curdling ferment is as follows:[2] Ten c.cm. of gastric filtrate are exactly neutralized with a $\frac{1}{10}$ normal NaOH solution, and 10 c.cm. of neutral milk are added, and the mixture placed in an incubator at 38° C. If the rennet-ferment is present, a casein-coagulum is formed in from ten to fifteen minutes.

The detection of the rennet-zymogen is as follows:

To 10 c.cm. of gastric filtrate, made slightly alkaline, 2 c.cm. of a 1 per cent. solution of calcium chlorid are added, and then 10 c.cm. of milk, and the mixture placed in the thermostat.

If the rennet-zymogen is present, a heavy cake of casein is precipitated in a few minutes.

The quantitative estimation of the milk-curdling ferment is made as follows: A part of the gastric filtrate is exactly neutralized, and portions are diluted with distilled water ($\frac{1}{10}$, $\frac{1}{15}$, $\frac{1}{20}$, etc.). Five c.cm. of each of these portions are placed in beakers, 5 c.cm. of neutral milk are added, and the mixtures placed in the thermostat. It can thus easily be determined at which dilution the ferment is no longer active.

As to the quantitative estimation of the rennet-

[1] Hammerschlag: Ueber eine neue Methode zur quantitative Pepsinbestiumung. Internationale klin. Rundschau, Jahrg. viii., Sept. 1894, No. 59.

[2] Loc. cit., S. 188.

zymogen, a part of the gastric filtrate is made slightly alkaline and portions diluted ($\frac{1}{10}$, $\frac{1}{15}$, $\frac{1}{20}$, $\frac{1}{25}$, $\frac{1}{30}$, $\frac{1}{35}$, etc.). To 5 c.cm. of each of these portions 1 c.cm. of a 1 per cent. solution of calcium chlorid is added, and 5 c.cm. of milk. The dilution can thus be determined at which the rennet-zymogen is no longer active.

By means of this method Boas has arrived at the following conclusions:

1. In spite of the absence of free hydrochloric acid, the rennet-ferment may still be present, but only in small amount, in dilutions of from $\frac{1}{10}$ to $\frac{1}{20}$.

2. In the absence of free hydrochloric acid the zymogen may be present in normal amount, even in dilutions of from $\frac{1}{100}$ to $\frac{1}{150}$. The repeated demonstration of the normal proportion of the zymogen proves with great certainty that an organic gastric disorder is not present, and that there is either a neurosis or a secondary gastric congestion.

3. The zymogen may be diminished one-half. This is most frequently due to a catarrh of moderate intensity. The more nearly the zymogen reaches the normal the greater is the probability of entire recovery under proper treatment.

4. If the labzymogen is much diminished in quantity, *e. g.*, $\frac{1}{10}$, or entirely absent, there is always a severe and incurable catarrh, which may be primary, or due to another disease, as carcinoma, amyloid degeneration, etc.

5. In the conditions represented by 1, 2, and 3, the secretion of hydrochloric acid may be increased by proper treatment. In the condition represented

by 4, there is but little hope of renewing the secretion of hydrochloric acid.

During several years I have as a routine practice made examinations of the gastric contents obtained one hour after an Ewald test-breakfast, to determine the activity of the milk-curdling ferment and its zymogen. It is to the result of these examinations that I wish to call attention.

Of the cases selected from many hundreds only those have been tabulated in which at least three examinations were made. In order to reduce the tables still more I have omitted many cases giving results exactly similar to others already given.

In Table I are represented the cases in which there was a normal percentage of free hydrochloric acid. There are here tabulated the results obtained from three normal cases, three cases of atony, a case of simple dilatation, and three cases of nervous dyspepsia. In order to make the tables more intelligible, the points of dilution at which the milk-curdling ferment and its zymogen were still present are placed in full numbers in this as well as in the following tables. They should, therefore, read $\frac{1}{12}$ for 12; $\frac{1}{50}$ for 50, etc.

It is seen that the degree to which the milk-curdling ferment may be diluted is much less than that of its zymogen, for, while the former is never present in dilutions less than $\frac{1}{40}$, its zymogen may at times be still distinctly recognized in dilutions of $\frac{1}{150}$.

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TABLE I.—CASES WITH NORMAL PERCENTAGE OF FREE HYDROCHLORIC ACID.

Number of examination.	Name.	Disease.	Total acidity.	Per cent. of free HCl.	Milk-curdling ferment.	Milk-curdling zymogen.
1	F. J.	Normal	50	0.175	12	150
2	...	.....	64	0.161	15	150
3	...	.....	48	0.148	28	100
4	E. F.	Normal	42	0.139	24	75
5	..	.....	46	0.158	22	40
6	...	.....	54	0.149	22	50
7	J. M.	Normal	55	0.169	18	80
8	...	.....	51	0.160	40	25
9	...	.....	58	0.154	32	150
10	F. B.	Atony	40	0.143	10	125
11	...	.....	44	0.145	34	75
12	...	.....	42	0.141	15	100
13	M. W.	Atony	48	0.159	18	150
14	...	.....	50	0.151	15	75
15	...	.....	44	0.139	40	50
16	L. P.	Atony	50	0.148	38	70
17	...	.....	48	0.139	12	125
18	...	.....	55	0.152	40	50
19	H. K.	Simple dilatation . .	68	0.169	12	90
20	...	.....	62	0.165	10	100
21	...	.....	63	0.160	35	55
22	H. T.	Nervous dyspepsia . .	48	0.142	40	35
23	...	.....	44	0.149	12	90
24	...	.....	40	0.134	16	100
25	K. S.	Nervous dyspepsia . .	42	0.140	15	125
26	...	.....	44	0.139	40	35
27	...	.....	48	0.134	20	100
28	H. T.	Nervous dyspepsia . .	50	0.138	18	75
29	...	.....	46	0.141	12	150
30	...	.....	40	0.135	24	70

TABLE II.—CASES WITH INCREASED AND DIMINISHED PERCENTAGE OF FREE HYDROCHLORIC ACID.

Number of examination.	Name.	Disease.	Total acidity.	Per cent. of free HCl.	Milk-curdling ferment.	Milk-curdling zymogen.
1	B. C.	Supersecretion . .	89	0.305	40	50
2	...	.....	86	0.302	35	45
3	...	.....	82	0.297	40	25
4	J. A.	Supersecretion . .	98	0.301	32	25
5	...	.....	96	0.302	30	55
6	...	.....	82	0.289	25	10
7	D. W.	Superacidity; ulcer .	84	0.286	15	25
8	...	.....	89	0.305	25	20
9	...	.....	90	0.301	20	30
10	P. J.	Superacidity; nerv. dysp.	90	0.302	20	30
11	...	.....	89	0.302	20	35
12	...	.....	88	0.298	15	30
13	G. A.	Superacidity; nerv. dysp.	88	0.301	25	15
14	...	.....	92	0.302	20	20
15	...	.....	98	0.310	15	25
16	F. L.	Superacidity; nerv. dysp.	92	0.301	15	20
17	...	.....	89	0.295	20	55
18	...	.....	86	0.302	35	45
19	F. K.	Subacidity; nerv. dysp. .	30	0.088	35	30
20	...	.....	28	0.084	20	35
21	...	.....	26	0.082	10	40
22	J. D.	Subacidity; nerv. dysp. .	24	0.081	25	40
23	...	.....	28	0.086	25	20
24	...	.....	29	0.089	10	25
25	S. F.	Chronic gastritis . .	34	0.102	20	50
26	...	.....	26	0.091	12	40
27	...	.....	24	0.084	14	35
28	K. M.	Chronic gastritis . .	34	0.096	18	25
29	...	.....	38	0.099	12	20
30	...	.....	36	0.104	14	25
31	L. P.	Chronic gastritis . .	37	0.098	10	25
32	...	.....	34	0.098	14	20
33	...	.....	32	0.092	20	15

In Table II are represented the cases with increased or diminished percentage of free hydrochloric acid. There are two cases of supersecretion, a case of superacidity due to ulcer, two cases of nervous dyspepsia, all with marked superacidity, besides three cases of subacidity (nervous dyspepsia), and three cases of chronic gastritis with subacidity.

The milk-curdling ferment is not found in dilutions beyond $\frac{1}{40}$; its zymogen may be present in dilutions of from $\frac{1}{50}$ to $\frac{1}{10}$.

In Tables III A and III B are found the cases of nervous dyspepsia and secondary gastric disturbances, with entire absence of free hydrochloric acid. It is seen that while the ferment may be markedly diminished, its zymogen may still be present in dilutions of from $\frac{1}{150}$ to $\frac{1}{60}$.

TABLE III.—GASTRIC DISTURBANCES IN WHICH THERE IS AN ABSENCE OF FREE HYDROCHLORIC ACID.

A. Nervous Dyspepsia.

No. of examination.	Name.	Total acidity.	Milk-curdling ferment.	Milk-curdling zymogen.	No. of examination.	Name.	Total acidity.	Milk-curdling ferment.	Milk-curdling zymogen.
1	K. T.	8	5	100	7	P. L.	8	15	80
2	...	7	8	75	8	...	12	15	110
3	...	12	5	60	9	...	10	10	130
4	J. S.	12	12	70	10	F. M.	20	12	100
5	...	14	5	80	11	...	22	5	80
6	...	20	5	150	12	...	18	8	60

B. Secondary Gastric Disturbances (Hyperemic Condition of Stomach).

No. of examination.	Name.	Primary disease.	Total acidity	Milk-curdling ferment.	Milk-curdling zymogen.
1	K. F.	Pulmonary tuberculosis	8	5	75
2	...	.....	12	8	80
3	...	.....	10	5	75
4	L. F.	Pulmonary tuberculosis	15	5	65
5	...	.....	14	10	75
6	...	.....	8	8	100
7	P. M.	Pulmonary tuberculosis	12	5	120
8	...	.....	10	12	80
9	...	.....	14	5	65
10	J. P.	Heart-disease . .	20	16	150
11	...	.....	12	5	75
12	...	.....	10	8	150
13	J. F.	Heart-disease . .	14	10	75
14	...	.....	10	10	100
15	...	.....	14	15	80
16	W.S.	Heart-disease . .	18	14	100
17	...	.....	9	8	150
18	...	.....	15	10	80

C. Chronic Gastritis.

No. of examination.	Name.	Total acidity.	Milk-curdling ferment.	Milk-curdling zymogen.	No. of examination.	Name.	Total acidity.	Milk-curdling ferment.	Milk-curdling zymogen.
1	B. H.	8	5	20	16	M. W.	0	0	5
2	...	10	8	25	17	...	0	0	5
3	...	12	10	10	18	...	0	0	7
4	G. F.	12	5	5	19	O. S.	0	0	10
5	...	10	10	25	20	...	0	0	5
6	...	14	12	15	21	...	0	0	5
7	S. S.	8	5	30	22	J. R.	0	0	7
8	...	9	5	10	23	...	0	0	5
9	...	10	5	25	24	...	0	0	7
10	B. W.	10	8	20	25	..	0	0	5
11	...	8	10	25	26	...	0	0	5
12	...	6	10	30	27	...	0	0	3
13	F. W.	4	12	25					
14	...	6	5	10					
15	...	4	5	5					

In Table III C we have represented cases of chronic gastritis. The milk-curdling ferment is much diminished (even to o), the zymogen between $\frac{1}{80}$ and $\frac{1}{8}$. In cases of carcinoma, Table III D, the milk-curdling ferment is diminished, its zymogen reduced to from $\frac{1}{40}$ to $\frac{1}{15}$.

D. Carcinoma.

No. of examination.	Name.	Total acidity.	Milk-curdling ferment.	Milk-curdling zymogen.	No. of examination.	Name.	Total acidity.	Milk-curdling ferment.	Milk-curdling zymogen.
1	D. B.	8	10	20	13	F. L.	15	5	35
2	...	4	8	20	14	...	14	10	40
3	...	6	10	15	15	...	12	10	35
4	A. C.	10	15	25	16	O. M.	12	10	35
5	...	14	5	30	17	...	8	12	40
6	...	12	12	35	18	...	6	15	25
7	F. G.	14	8	25	19	L. T.	8	15	30
8	...	12	5	25	20	...	8	15	35
9	.	14	5	20	21	...	10	8	40
10	B. T.	13	5	20	22	S. L.	12	8	35
11	...	19	8	25	23	...	20	5	35
12	...	21	8	25	24	...	8	5	30

We have thus shown that in conditions in which the free hydrochloric acid is absent, but in which there is no pathologic change in the stomach, such as in nervous dyspepsia and secondary gastric catarrh, the zymogen is still present in dilutions ranging between $\frac{1}{150}$ and $\frac{1}{60}$. In those conditions, however, in which there are structural changes in the gastric mucous membrane, such as in chronic gastric catarrh and carcinoma, the zymogen is markedly diminished ($\frac{1}{80}$–$\frac{1}{8}$), depending upon the

severity of the disease. The more nearly the zymogen reaches the zero-point the greater the destruction of the gastric mucous membrane, and the less the chance for complete recovery.

Our conclusions may be summed up as follows:

1. Under normal conditions the milk-curdling ferment may be present in dilutions up to $\frac{1}{40}$, the zymogen up to $\frac{1}{150}$.

2. In those cases in which there is a normal or diminished percentage of free hydrochloric acid, the milk-curdling ferment and its zymogen may be present in normal quantities or may be markedly diminished. Their estimation, therefore, in these cases is of little value.

3. The estimation of the milk-curdling ferment and its zymogen is of great diagnostic as well as prognostic importance in those cases of gastric disorder accompanied by an entire absence of free hydrochloric acid. In these cases (chronic gastritis or carcinoma) there is marked diminution of the zymogen ($\frac{1}{40}$–0), depending upon the severity and extent of the disease. In cases of nervous dyspepsia, as well as in secondary catarrh, the zymogen is present in normal proportions in dilutions of from $\frac{1}{150}$ to $\frac{1}{60}$. We can, therefore, readily determine whether there is actual disease of the gastric mucous membrane or simply a nervous or congestive condition.

4. In those cases in which there is an absence of free hydrochloric acid, and in which the labzymogen falls between $\frac{1}{60}$ and $\frac{1}{40}$, it is impossible to determine at once whether there is a catarrhal condition or nervous dyspepsia present. Several examinations

must be made to determine whether the labzymogen ranges above $\frac{1}{60}$ or below $\frac{1}{40}$.

5. In cases of chronic gastritis the examination for the labzymogen is of considerable prognostic importance. In those cases in which the labzymogen is diminished from $\frac{1}{15}$ to 0 there is no chance of recovery; in those in which it is diminished from $\frac{1}{40}$ to $\frac{1}{30}$ there is a possibility that judicious treatment may result in recovery.